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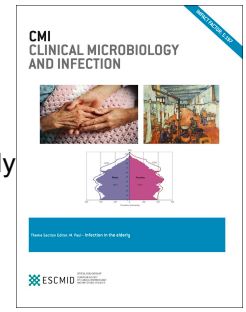

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Female gender is associated with "long COVID" syndrome: a prospective cohort study

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Long COVID

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Key words: COVID-19, long COVID, post-COVID syndrome, Gender

Abstract

Objective

We explored the association between female gender and "long COVID" syndrome, defined as persistence of physical and/or psychological symptoms for more than 4 weeks after recovery from acute COVID-19 disease. Secondary aim was to identify predictors of "long COVID" syndrome by multivariable logistic regression analysis.

Methods

This is a single-centre prospective cohort study conducted at San Paolo Hospital in Milan, Italy. We enrolled adult patients that were evaluated at the post-COVID outpatient service of our Infectious Diseases Unit between April 15th 2020 and December 15th 2020. Participants were individuals who had clinically recovered from COVID-19 and in whom virological clearance had occurred. Previous infection by SARS-CoV-2 was microbiologically documented by positivity at reverse-transcriptase polymerase chain reaction (RT-PCR) assay of nasopharyngeal swab. All enrolled patients underwent blood tests and a comprehensive medical examination at follow-up. Individuals were interviewed about resolved and persisting symptoms and were asked to fill in two questionnaires to allow assessment of the Hospital Anxiety and Depression symptoms (HADS) score and of the Impact of Event Scale–Revised (IES-R) score.

Results

A total of 377 patients were enrolled in the study. The median time from symptom onset to clinical recovery and virological clearance was 79 (IQR 69-102) and 56 (IQR 47-74) days, respectively. A diagnosis of "long COVID" syndrome was made in 260/377 (69%) patients. The most common reported symptoms were fatigue (149/377, 39.5%), exertional dyspnoea (109/377, 28.9%), musculoskeletal pain (80/377, 21.2%) and "brain fog" (76/377, 20.2%). Anxiety symptoms were ascertained in 71/377 (18.8%) individuals, whereas 40/377 (10.6%) patients presented symptoms of depression. Post-traumatic stress disorder (defined by a pathological IES-R score) was diagnosed in one-third of patients (85/275, 31%). Female gender was independently associated with "long COVID" syndrome at multivariable analysis (AOR 3.3 versus males, 95%CI 1.8-6.2, p<0.0001). Advanced age (AOR 1.03 for 10 years older, 95%CI 1.01-1.05, p=0.01) and active smoking (AOR 0.19 for former smokers *vs* active smokers, 95%CI 0.06-0.62, p=0.002) were also associated with a higher risk of "long COVID", while no association was found between severity of disease and "long COVID" (AOR 0.67 for Continuous Positive Airway Pressure [CPAP]/Non-Invasive Mechanical Ventilation [NIMV]/Orotracheal Intubation [OTI] *vs* no 02 therapy, 95%CI 0.29-1.55, p=0.85).

Conclusion

Factors that were found to be associated with a higher risk of developing “long COVID” syndrome were female gender and active smoking, but not severity of the acute disease. Individuals affected by SARS-CoV-2 infection with the aforementioned features should be early identified and involved in follow-up programs.

Introduction

Over half of individuals affected by COVID-19 reports persisting symptoms after recovery from acute illness. These symptoms can endure up to 6-7 months and even longer [1-5]. Since the persistence of symptoms represents a health concern, this phenomenon was given the name of "long COVID" syndrome. In the UK, the National Institute for Health and Care Excellence (NICE) defined "long COVID" as the persistence of symptoms for more than 4 weeks after the onset of acute illness [6-8]. Ongoing physical symptoms include fatigue, muscle weakness, shortness of breath or cough, joint or chest pain, as well as difficulty in concentration and memory disorders; the latter two symptoms re-enter in a broader state of diminished mental capacity that may be described as "brain fog" [1, 4, 9-11]. As far as psychological manifestations are concerned, the most frequent are post-traumatic stress disorder (PTSD), depression, anxiety, obsessive-compulsive traits and insomnia [1, 12-15]. Previous studies have not distinctly identified predictors of "long COVID" syndrome; factors found to have a correlation with "long COVID" so far were: increased length of acute illness, longer time from symptom onset to virological clearance and comorbidities [11, 16]. Increased COVID-19 severity and female gender as predicting factors are instead not equivocal findings [1, 4, 5, 8, 13, 17-20]. Nevertheless, what emerges from preliminary data is the negative impact of "long COVID" on quality of life and its effects on patient work ability and social relationships [1, 4, 19]. Given the extent of such phenomenon, healthcare public resources will be utilised to face the issue even when the COVID-19 emergency status will end. It is therefore necessary to better understand the characteristics of "long COVID", its predictors and possible long-term sequelae. Furthermore, the likelihood of presenting ongoing symptoms might differ according to several factors (e.g. socioeconomic status or cultural background) that should therefore be identified.

We thus investigated the incidence of physical and/or psychological symptoms characterising the "long COVID" syndrome in female gender and to possibly identify predictors of "long COVID".

Methods

Study design and Ethics

This is a single-centre prospective cohort study conducted at San Paolo Hospital in Milan, Italy. Informed consent from all participants was obtained. The study was approved by the Ethical Committee Area 1, Milan (2020/ST/049 and 2020/ST/049_BIS, March 11th 2020).

Study population

We enrolled adult patients that were evaluated at the post-COVID outpatient clinic, that was set up starting from April 2020, of our Infectious Diseases Unit between April 15th 2020 and December 15th 2020. Participants were patients who had been hospitalised for COVID-19 at San Paolo and San Carlo Hospitals in Milan and had been discharged between March 1st 2020 and November 1st 2020. Previous infection by SARS-CoV-2 was microbiologically documented by positivity at reverse-transcriptase polymerase chain reaction (RT-PCR) assay of nasopharyngeal swab. At the time of hospital discharge, patients were given an appointment for a follow-up visit in the outpatient clinic.

Not included in the study were patients who died during hospitalisation or after discharge, those who were not given a follow-up appointment because declined to participate or were unable to reach the hospital, and those who missed their appointment and were therefore lost to follow-up.

Patients who had been hospitalised for other medical issues and had a positive SARS-CoV-2 nasopharyngeal swab but remained asymptomatic for COVID-19, were also excluded from the study.

Follow-up implied:

-Repetition of nasopharyngeal swab for patients who were discharged with the indication to self-isolate since their upper respiratory swab was still positive at the time of discharge

-Blood tests and comprehensive medical visit at 1 to 3 months and later at 6 months after virological clearance (namely a negative nasopharyngeal swab).

This study reports data about the first follow-up assessment at 1-3 months after virological clearance.

A trained nurse was in charge of taking blood samples, whereas a team of Infectious Diseases specialists and clinical psychologists were involved in visiting and interviewing participants. Patients were asked to self-report a detailed list of symptoms and signs, specifying whether they had resolved or were still ongoing. Moreover, they were invited to fill in two questionnaires: the Hospital Anxiety and Depression Symptoms (HADS)[21] and the Impact of Event Scale–Revised (IES-R)[22]. A comprehensive physical examination was performed by trained Infectious Diseases specialists. Clinical psychologists, on the other hand, revised the questionnaires and identified symptoms of anxiety or depression and PTSD, by evaluating HADS and IES-R scores respectively.

Aims of the study

Primary aim of the study was to assess the incidence of "long COVID" syndrome in females. Secondary aim was to identify predictors of "long COVID" by multivariable logistic regression analysis.

Study procedures

Data of the acute phase of illness were collected. Disease severity was defined by the highest level of respiratory support required during hospitalisation (no oxygen therapy required, low- or high- flow oxygen therapy, Continuous Positive Airway Pressure [CPAP], Non-Invasive Mechanical Ventilation [NIMV] and Orotracheal Intubation [OTI]). Treatments for COVID-19 included antiviral agents (remdesivir [RDV] or HIV protease inhibitors), hydroxychloroquine alone or in association with azithromycin, corticosteroids and immunomodulatory drugs (IL-6 receptor antagonists and JAK-STAT inhibitors). At follow-up, all patients underwent routine blood tests and a medical evaluation. All patients were asked about the following signs and symptoms: anosmia, dysgeusia, fever, gastro-intestinal symptoms, rest or exertional dyspnoea, fatigue, musculoskeletal pain, muscle weakness, "brain fog" manifestations (namely difficulties in attention or concentration and memory disorders). HADS [21] was intended to measure anxiety and depression symptoms, whereas IES-R was used as a screening tool of PTSD [22]. A total HADS score higher than 8 denoted considerable symptoms of

anxiety and depression, while a IES-R score above 33 was interpreted as highly suggestive for PTSD. "Long COVID" was defined as the persistence of ≥1 physical and/or psychological symptoms at follow-up [7].

Statistical analyses

Data were presented as median and interquartile range (IQR) for quantitative parameters and absolute numbers and percentages for categorical variables. A Venn diagram was used to show possible correlations among physical and psychological symptoms characterizing "long COVID". Comparison between the group of patients diagnosed with "long COVID" and that of patients who did not and between females and males was investigated by Mann-Whitney test and Chi-square or Fisher's exact test. The association between female gender and "long COVID" was analysed by fitting a multivariable logistic regression analysis, adjusting for possible confounders (age, severity of disease, length of hospital stay [LOS], comorbidities, Body Mass Index [BMI], smoking and time to virological clearance). Missing data were handled by the missing-indicator method. Statistical analyses were performed with STATA software, version 14.0.

Results

Figure 1 depicts the study flowchart. 492 patients discharged from San Paolo and San Carlo Hospitals in the aforementioned time period were given an appointment for a follow-up evaluation at the post-COVID outpatient clinic. 115/492 (23%) patients missed their visit, thus the study includes a total of 377/492 individuals, namely 77% of those who were given an appointment. Demographic and clinical characteristics of patients who missed the follow up appointment are presented in the Supplementary Table 1.

The follow-up examination was done at a median of 102 (IQR 86-126) days from acute symptom onset, a median of 79 (IQR 69-102) days from clinical recovery and a median of 56 (IQR 47-74) days from virological clearance. Baseline demographic and clinical features of the enrolled patients are

shown in Table 1. Females accounted for 137/377 (36%) of all patients; median age was 57 (IQR 49-68) years. The most frequent acute symptoms were fever (280/377, 74%), cough (198/377, 53%), dyspnoea (161/377, 43%) and fatigue (59/377, 16%). 346/377 (92%) presented interstitial pneumonia at chest X-ray or CT scan at the time of hospital admission. One-third of patients received CPAP or NIMV (115/377, 31%) during hospitalisation, whereas only 30/377 (8%) patients underwent OTI.

Women were less frequently active smokers as compared to men. Furthermore, overall patients of female gender presented a milder form of disease, estimated as a lower proportion of OTI and a shorter LOS (Table 2). Blood tests at follow-up showed no significant alterations: C-reactive protein (CRP) and complete blood count (CBC) were normal for all patients, while D-dimer was still out of range in 28/377 (8%) patients without however any correlation with organ dysfunction (median pathological D-dimer was 399, IQR 310.7-579.2 ng/mL). In all 28 patients with elevated D-dimer at follow-up, levels were lower as compared to the acute phase of illness; however, 2 of these patients presented persisting dyspnoea and therefore underwent chest CT scan with contrast medium that excluded pulmonary thromboembolism.

"Long COVID" was observed in 260/377 (69%) patients; interestingly, 112/137 (81.7%) females presented "long COVID" syndrome. Within "long COVID" patients, 97/260 (37.3%) participants had only one persisting symptom, 84/260 (32.3%) had two persisting symptoms and 79/260 (30.4%) had three or more persisting symptoms. More specifically, a significant number of patients (142/260, 55%) reported ongoing physical symptoms only, 100/260 (38%) complained both physical and psychological symptoms, while 18/260 (7%) presented psychological distress solely at follow-up (Supplementary Figure 1a). Physical and psychological manifestations were similarly represented in both genders (Supplementary Figure 1b and 1c).

The most common physical symptoms characterising "long COVID" were fatigue (149/377, 39.5%), exertional dyspnoea (109/377, 28.9%), musculoskeletal pain (80/377, 21.2%) and "brain fog" (76/377, 20.2%) (Table 3a).

As far as psychological sequelae are concerned, manifestations of anxiety were the most frequent (71/377, 18.8%), while depression symptoms were present in 40/377, 10.6% of patients (although 38 patients did not complete HADS questionnaire being non-Italian speakers) (Table 3b). IES-R was available in an unselected sub-group of patients (275/377, 73% patients): in one-third (85/275, 31%) of cases the IES-R score resulted pathological (according to the aforementioned definition), possibly suggesting the presence of PTSD (Table 3b). Interestingly, women were characterized by a higher proportion of most physical symptoms and all psychological symptoms, compared to males (Table 3).

At multivariable logistic regression analysis, female gender was associated with a 3-fold higher risk of "long COVID", also after adjustment for age, severity of illness, LOS, comorbidities, smoking, BMI and time from symptoms onset to virological clearance (Table 4). Other independent predictors of "long COVID" were advanced age and active smoking. Severity of disease, LOS and time to virological clearance were not associated with "long COVID" at univariable and multivariable analyses (Table 4).

Discussion

The American Center for Disease Control (CDC) and the British NICE have identified symptoms that could persist for weeks or even months after recovery from COVID-19. How long these symptoms could endure for, possible risk factors for their persistence and the predisposing patient features are all aspects to be further elucidated [6-8, 17]. Our study reveals that:

(i) The most common symptoms characterizing "long COVID" were both physical and psychological;

(ii) Females had a 3-fold higher risk of being diagnosed with "long COVID";

(iii) severity of disease and time to virological resolution were not associated with "long COVID";

The high incidence of "long COVID" within our cohort was similar to that previously reported: more than half of patients reported symptoms lasting more than two months after symptom onset [4, 5, 16,

18, 19]. Some symptoms are also commonly seen in other viral illnesses and both psychical and psychological sequelae have been described in MERS and SARS [12, 23-29].

Literature data are not equivocal about the association between females and "long COVID": some preliminary studies have shown an increased prevalence of fatigue [24] or other symptoms among women [1, 5, 13, 16], while in other studies no gender association was found [4, 18-20]. Differences in ethnicity, living country and possibly socio-economic status might explain such contrasting results. Hormones may play a role in perpetuating the hyperinflammatory status of the acute phase even after recovery [30, 31] and a stronger IgG antibodies production in females in the early phase of disease has been reported; this could turn out in a more favourable outcome in women [32], but might play a role as well in perpetuating disease manifestations. Furthermore, we might hypothesise that women are in general more attentive to their body and related distress.

Advanced age was associated with ongoing fatigue and musculoskeletal pain, or impairment in pulmonary functions, reflecting a decline in organ function and a slower ability to recover [1, 16]. As far as our finding that reported a weak association between obesity and "long COVID" is concerned, this was not confirmed in a previous study that however focused on persisting fatigue solely [24]. Increased inflammation, defective adaptative immune responses, endothelial dysfunction and coagulation-related disorders are all phenomena that have been well described in obesity and might represent a plausible explanation to the link we observed between high BMI and "long COVID" [33]. Finally, the relationship between smoking and "long COVID" has not been previously explored; the most frequent symptom reported by smokers was however shortness of breath, which possibly reflects an underlying pulmonary dysfunction.

We were expecting to find an association between severity of disease and residual symptoms, as reported by previous studies [1]. However, "long COVID" has been previously described also in not hospitalised patients diagnosed with a mild, self-limiting disease [6, 16-18, 24].

Our study has some limitations: a possible selection bias due to losses to follow-up; the limited sample size for patients with severe disease and females; the lack in validated tools to assess dyspnoea and fatigue and in information about the presence of the symptoms before the onset of acute infection. The possible association between female gender and "long COVID" should be confirmed in larger populations and by means of a longer follow-up. The evaluation of our patients at six months still needs to be completed and this will certainly allow us to collect further data and build stronger evidence.

In conclusion, in our setting "long COVID" was a frequent long-term complication of COVID-19 and was diagnosed more frequently in women as compared to men. It was also commonly seen in patients who recovered from a mild disease form. Follow-up outpatient services are therefore needed in order to manage this syndrome and to better understand the possible association between symptoms and residual organ impairment, and their impact on patient quality of life.

Transparency declaration

Conflict of interest: Authors don't have conflicts of interests to declare for this work.

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Contribution: F Bai wrote the manuscript. D Tomasoni created the dataset, D Tomasoni and F Bai performed statistical analyses. F Bai, D Tomasoni, C Falcinella, D Barbanotti, R Castoldi, G Mulè,

M Augello, D Mondatore, M Allegrini, A Cona, D Tesoro, G Tagliaferri, O Viganò, E Suardi, C Tincati and T Beringheli followed up patients, contributed to acquisition of data and revised the article. A Tavelli and S Terzoni contributed to analyses and interpretation of data. G Marchetti and A d'Arminio Monforte contributed to the design of the study and revised the article. C Luridiana, K Piscopo, E Vegni performed psychological evaluations and revised the final version of the manuscript. All the authors have read and approved the final version of the paper.

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Table 1 Demographic and clinical characteristics of enrolled patients according to "long COVID" syndrome

Parameters	Total population (N 377)	No long COVID (N 117)	Long COVID (N 260)	p values
Age, years*	57 (49-68)	56 (42-64)	58 (51-69)	**0.01**
Gender, males°	240 (63.7%)	92 (78.6%)	148 (56.9%)	**<0.0001**
Comorbidities°	164 (43.5%)	45 (38.5%)	119 (45.8%)	0.241
Smoking°:				**0.046**
Never	216 (57.3%)	71 (60.7%)	145 (55.8%)	
Ex smokers	20 (5.3%)	9 (7.7%)	11 (4.2%)	
Active smokers	131 (34.7%)	30 (25.7%)	101 (38.8%)	
Unknown	10 (2.7%)	7 (5.9%)	3 (1.2%)	
Ethnicity°:				0.579
White/Caucasian	252 (66.8%)	82 (70.1%)	170 (65.4%)	
Arabic	16 (4.2%)	6 (5.1%)	10 (3.8%)	
Black	2 (0.5%)	0	2 (0.8%)	
Asian	7 (1.9%)	0	7 (2.7%)	
Hispanic	33 (8.7%)	9 (7.7%)	24 (9.2%)	
Unknown	67 (17.8%)	20 (17.1%)	47 (18.1%)	
During hospitalization				
Symptoms at admission°:				
Fever	280 (74.3%)	96 (82.1%)	184 (70.8%)	**0.023**
Cough	198 (52.5%)	67 (57.3%)	131 (50.4%)	0.360
Sore throat	7 (1.9%)	3 (2.6%)	4 (1.5%)	0.501
Chest pain	16 (4.2%)	6 (5.1%)	10 (3.8%)	0.593
Myalgia-arthralgia	28 (4.8%)	10 (8.5%)	18 (6.9%)	0.422
Fatigue	59 (15.6%)	20 (17.1%)	39 (15%)	0.732
Headache	11 (2.9%)	4 (3.4%)	7 (2.7%)	0.754
Dyspnoea	161 (42.7%)	46 (39.3%)	115 (44.2%)	0.458
Vomiting/nausea	25 (6.6%)	9 (7.7%)	16 (6.2%)	0.526
Anosmia/dysgeusia	24 (6.4%)	9 (7.7%)	15 (5.8%)	0.478
Pneumonia:				0.372
Absence of pneumonia	31 (8.2%)	9 (7.7%)	22 (8.5%)	
Pneumonia	346 (91.8%)	108 (92.3%)	238 (91.5%)	
Oxygen therapy°:				0.879
No 02 therapy	35 (9.3%)	13 (11.1%)	22 (8.4%)	
Low/high flows systems	197 (52.2%)	60 (51.3%)	137 (52.7%)	
CPAP, NIV	115 (30.6%)	35 (29.9%)	80 (30.8%)	
OTI	30 (7.9%)	9 (7.7%)	21 (8.1%)	
Length of hospital days (LOS)*	11 (6-20)	10 (6-16)	12 (7-21)	0.232
Treatments for COVID-19:				
Antivirals (RDV or PI)	92 (22.4%)	30 (25.6%)	62 (23.8%)	0.746
HQC +/- Azithromycin	260 (68.9%)	83 (70.9%)	177 (68.1%)	0.661
Steroid	42 (11.1%)	14 (11.9%)	28 (10.8%)	0.798
immunomodulators	28 (7.4%)	11 (9.4%)	17 (6.5%)	0.334

Obesity° BMI <30 BMI ≥30 Unknown	 227 (60.2%) 136 (36.1%) 14 (3.7%)	 79 (67.5%) 31 (26.5%) 7 (6%)	 148 (61.7%) 105 (40.4%) 7 (2.7%)	**0.021**
Follow-up visit				
Time since clinical recovery, days*	79 (69-102)	81 (69-108)	79 (69-97)	0.599
Time since virological clearance, days*	56 (47-74)	56 (46-79)	56 (47-74)	0.981
Time since symptoms onset, days*	102 (86-126)	101 (85-131)	103 (86-124)	0.664
Time from symptoms onset to virological clearance, days*	44 (37-53)	43 (35-50)	44 (38-55)	0.081
Blood exams at follow-up				
WBC, x10^3/mmc*	6.07 (5.11-7.07)	5.89 (5.19-7.22)	6.08 (5.1-6.99)	0.770
Hb, g/dL*	14.1 (13.2-15)	14.6 (13.7-15.5)	13.9 (12.8-14.8)	**<0.0001**
PLT, x10^3/mmc*	243 (203-281)	237 (208-272)	246 (202-288)	0.325
Lymphocytes, cell/mmc*	2.2 (1.81-2.6)	2.24 (1.87-2.7)	2.18 (1.79-2.57)	0.274
Creatinine, mg/dL*	0.8 (0.67-0.9)	0.8 (0.7-1)	0.8 (0.6-0.9)	0.129
GOT, UI/L*	27 (24-31)	25 (21-30)	26 (23-31)	0.198
GPT, UI/L*	21 (19-31)	20 (16-29)	20 (16-27)	0.571
LDH, UI/L*	196 (177-213)	188 (173-209)	196 (180-219)	0.247
CRP, mg/L*	5 (5-5.2)	5 (5-5.1)	5 (5-5.1)	0.484
D dimer, mg/L*	120 (81-199)	110 (78-177)	126 (87-204)	**0.04**

LEGEND

*Quantitative variables are presented as median, (Interquartile Range); °categorical variables are presented as absolute numbers, (percentages).

CPAP, Continuous Positive Airway Pressure; OTI, orotracheal intubation – NIV, non invasive ventilation

Antivirals include: PI, Protease Inhibitors (Darunavir/Cobicistat, Darunavir/Ritonavir, Lopinavir/Ritonavir) and RDV (Remdesivir). HQC, hydroxychloroquine.

WBC, White Blood Cells; Hb, Hemoglobin; PLT, Platelet count; CRP, C Reactive Protein; GOT, Serum Glutamic Oxalacetic Transaminase; GPT, Serum Glutamic Pyruvic Transaminase; LDH, lactate dehydrogenase)

Table 2 Comparison between males and females

Parameters	Females (N 137)	Males (N 240)	p values
Age, years*	58 (50-68)	57 (49-67)	0.931
Comorbidities°	57 (41.6%)	107 (44.6%)	0.672
Smoking°:			**0.008**
Never	94 (68.6%)	122 (50.8%)	
Ex smokers	10 (7.3%)	10 (4.2%)	
Active smokers	31 (22.6%)	100 (41.7%)	
Unknown	2 (1.5%)	8 (3.3%)	
Ethnicity°:			0.691
White/Caucasian	90 (18.2%)	162 (67.5%)	
Arabic	6 (4.4%)	10 (4.2%)	
Black	2 (1.5%)	0	
Asian	2 (1.5%)	5 (2.1%)	
Hispanic	12 (8.8%)	21 (8.7%)	
Unknown	25 (18.2%)	42 (17.5%)	
During hospitalization			
Symptoms at admission°:			
Fever	96 (70.1%)	184 (76.7%)	0.215
Cough	61 (44.5%)	137 (57.1%)	**0.02**
Sore throat	3 (2.2%)	4 (1.7%)	0.91
Chest pain	10 (7.3%)	6 (2.5%)	**0.03**
Myalgia-arthralgia	16 (11.7%)	12 (5%)	0.392
Fatigue	21 (15.4%)	38 (15.8%)	0.914
Headache	4 (2.9%)	7 (2.9%)	0.999
Dyspnoea	54 (39.4%)	107 (44.6%)	0.452
Vomiting/nausea	11 (8.1%)	14 (5.8%)	0.49
Anosmia/dysgeusia	12 (8.8%)	12 (5%)	0.237
Pneumonia:			0.771
Absence of pneumonia	11 (8.1%)	20 (8.3%)	
Pneumonia	126 (91.9%)	220 (91.7%)	
Oxygen therapy°:			**0.009**
No 02 therapy	18 (13.1%)	17 (7.1%)	
Low/high flows systems	79 (57.7%)	118 (49.2%)	
CPAP, NIV	37 (27%)	78 (32.5%)	
OTI	3 (2.2%)	27 (11.2%)	
Length of hospital days (LOS)*	10 (6-16)	12 (7-21)	**0.033**
Treatments for COVID-19:			
Antivirals (RDV or PI)	33 (24.1%)	59 (24.5%)	0.892
HQC +/- Azithromycin	90 (65.7%)	170 (70.8%)	0.356
Steroid	13 (9.5%)	29 (12.1%)	0.576
Immunomodulating therapies	8 (5.8%)	20 (8.3%)	0.351
BMI, categories°			**0.003**
<18.5	5 (3.6%)	0	
18.5-24.99	31 (22.6%)	45 (18.7%)	
25-29.99	39 (28.5%)	107 (44.6%)	

≥30 Unknown	56 (40.9%) 6 (4.38%)	80 (33.3%) 8 (3.3%)	
Follow-up visit			
Time since virological clearance, days*	59 (49-77)	55 (46-72)	0.05
Time from symptoms onset to virological clearance, days*	45 (38-55)	44 (37-52)	0.471

LEGEND

*Quantitative variables are presented as median, (Interquartile Range); °categorical variables are presented as absolute numbers, (percentages).

CPAP, Continuous Positive Airway Pressure; OTI, orotracheal intubation – NIV, non invasive ventilation

Antivirals include: Darunavir/Cobicistat, Darunavir/Ritonavir, Lopinavir/Ritonavir, Remdesivir. HQC, hydroxychloroquine.

Table 3 Physical and psychological symptoms at follow-up

Parameter	Study population N 377	Males N 240	Females N 137	p value
a) PHYSICAL SYMPTOMS				
Anosmia				**0.017**
No, ever	179 (47.5%)	127 (52.9%)	52 (37.9%)	
Ongoing	18 (4.8%)	8 (3.3%)	10 (7.4%)	
Resolved	174 (46.2%)	104 (43.4%)	70 (51.1%)	
Unknown	6 (1.6%)	1 (0.4%)	5 (3.6%)	
Dysgeusia				**0.005**
No, ever	167 (44.3%)	120 (50.1%)	47 (34.3%)	
Ongoing	20 (5.3%)	8 (3.3%)	12 (8.7%)	
Resolved	184 (48.8%)	111 (46.2%)	73 (53.4%)	
Unknown	6 (1.6%)	1 (0.4%)	5 (3.6%)	
GI symptoms				**<0.001**
No, ever	201 (53.3%)	143 (59.6%)	58 (42.3%)	
Ongoing	6 (1.6%)	1 (0.4%)	5 (3.7%)	
Resolved	162 (43%)	91 (37.9%)	71 (51.8%)	
Unknown	8 (2.1%)	5 (2.1%)	3 (2.2%)	
Fever				0.966
No, ever	22 (5.8%)	14 (5.9%)	8 (5.8%)	
Ongoing	0	0	0	
Resolved	348 (92.3%)	223 (92.9%)	125 (91.2%)	
Unknown	7 (1.9%)	3 (1.2%)	4 (3%)	
Joint pain or myalgia				**<0.001**
No, ever	215 (57%)	156 (65%)	59 (43.2%)	
Ongoing	80 (21.2%)	30 (12.5%)	50 (36.4%)	
Resolved	74 (19.6%)	50 (20.8%)	24 (17.5%)	
Unknown	8 (2.1%)	4 (1.7%)	4 (2.9%)	
Rest Dyspnea				0.548
No, ever	103 (27.3%)	70 (29.2%)	33 (24.2%)	
Ongoing	24 (6.4%)	16 (6.7%)	8 (5.8%)	
Resolved	242 (64.2%)	150 (62.5%)	92 (67.1%)	
Unknown	8 (2.1%)	4 (1.6%)	4 (2.9%)	
Exertional Dyspnea				**0.036**
No, ever	87 (23.1%)	61 (25.5%)	26 (19%)	
Ongoing	109 (28.9%)	59 (24.6%)	50 (36.5%)	
Resolved	175 (46.4%)	117 (48.7%)	58 (42.3%)	
Unknown	6 (1.6%)	3 (1.2%)	3 (2.2%)	
Fatigue				**<0.001**
No, ever	67 (17.8%)	50 (20.9%)	17 (12.5%)	
Ongoing	149 (39.5%)	74 (30.8%)	75 (54.7%)	
Resolved	154 (40.8%)	113 (47.1%)	41 (29.9%)	
Unknown	7 (1.9%)	3 (1.2%)	4 (2.9%)	
Brain fog				**<0.001**
No, ever	274 (72.7%)	190 (79.2%)	84 (61.3%)	

Ongoing	76 (20.2%)	35 (14.6%)	41 (29.9%)	
Resolved	13 (3.4%)	6 (2.5%)	7 (5.1%)	
Unknown	14 (3.7%)	9 (3.7%)	5 (3.7%)	
Other				**0.003**
No, ever	300 (79.5%)	198 (82.6%)	102 (74.5%)	
Ongoing	58 (15.4%)	28 (11.7%)	30 (21.9%)	
Resolved	19 (5%)	14 (5.7%)	5 (3.6%)	
b) PSYCHOLOGICAL SYMPTOMS and PTSD				
IES-R, PTSD				**0.002**
Normal	190 (50.4%)	133 (55.4%)	57 (41.6%)	
Pathological	85 (22.5%)	43 (17.9%)	42 (30.6%)	
Unknown	102 (27.1%)	64 (26.7%)	38 (27.8%)	
HADS, depression symptoms				**0.009**
Normal	299 (9.3%)	198 (82.5%)	101 (73.7%)	
Pathological	40 (10.6%)	18 (7.5%)	22 (16.1%)	
Unknown	38 (10.1%)	24 (10%)	14 (10.2%)	
HADS, anxiety symptoms				**<0.001**
Normal	268 (71.1%)	185 (77.1%)	83 (60.6%)	
Pathological	71 (18.8%)	31 (12.9%)	40 (29.2%)	
Unknown	38 (10.1%)	24 (10%)	14 (10.2%)	

LEGEND

Parameters are presented as absolute numbers. percentages.

A) Physical symptoms
B) Psychological symptoms

GI. gastrointestinal symptoms; Other. chest pain. headache. constipation. tinnitus. insomnia. palpitations. NSTEMI. cough. sore throat.

IES-R. Impact of Event Scale - Revised for diagnosis of Post-Traumatic Stress Disorders; HADS. Hospital Anxiety and Depression Scale for diagnosis of anxiety and depression symptoms.

* p values <0.05 for comparison between males and females by Chi-square test.

Table 4 Factors associated with "long COVID" syndrome by fitting univariable and multivariable logistic regression analyses.

PARAMETERS	OR (95%CI)	p values	AOR (95%CI)	p values
Gender Male Female	 1 2.78 (1.68-4.62)	 **<0.0001**	 1 3.32 (1.78-6.17)	 **<0.0001**
Age, 10 years older	 1.03 (1.01-1.04)	 **0.001**	 1.03 (1.01-1.05)	 **0.01**
02 therapy, No 02 02 therapy low-high flows CPAP/NIV/IOT	 1 0.67 (0.38-1.19) 0.97 (0.55-1.71)	 0.17 0.91	 1 0.39 (0.19-0.82) 0.67 (0.29-1.55)	 0.44 0.85
LOS, Each day more	 1.01 (0.99-1.03)	 0.28	 0.998 (0.97-1.03)	 0.92
Comorbidities, No Yes	 1 1.35 (0.86-2.11)	 0.19	 1 1.05 (0.597-1.84)	 0.87
Smoking, Active Unknown Never Former	 1 0.13 (0.03-0.52) 0.61 (0.37-0.997) 0.36 (0.14-0.96)	 **0.004** **0.05** **0.04**	 1 0.16 (0.04-0.75) 0.56 (0.31-1.01) 0.19 (0.06-0.62)	 0.31 0.41 **0.002**
BMI, ≥30 Unknown <30	 1 0.29 (0.096-0.91) 0.55 (2.27-5.06)	 **0.03** **0.02**	 1 0.13 (0.30-0.53) 0.55 (0.31-0.98)	 **0.03** 0.28
Time from symptoms onset to virological clearance, each day more	1.01 (0.99-1.02)	0.64	0.99 (0.98-1.01)	0.47

LEGEND
AOR, adjusted odds ratio; 95%CI, 95% confidence interval. Outcome: long COVID syndrome.
Each variable is mutually adjusted.
*p values by Bonferroni correction.
CPAP, Continuous Positive Airway Pressure; OTI, orotracheal intubation; NIV, non invasive ventilation; LOS, length of hospital stay; BMI, Body Mass Index.

Figure 1 Study flow chart